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Abiotic Stress Tolerance in *Eleusine coracana* (L.) Gaertn .

By Manipulation of Tissue Culture Media

A synopsis

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BY:

SUMAN KRISHANIA

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DR.KALPANA AGARWAL

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Introduction

Scientific classification

Regnum: Vegetabile

Division: Angiospermae

Class: Monocotyledonae

Series: Glumaceae

family: Poaceae

Genus: ***Eleusine***

Species: ***coracana***

Binomial name

Eleusine coracana

Eleusine coracana

Abiotic stress is a major global problem limiting crop productivity. Stress factors are a serious problem limiting the yield potential of modern cultivars. Stress causes nutritional imbalances in the plant causing reduction in water uptake and toxicity, decreasing the product. Abiotic stresses like salinity, heavy metals and pesticides are the primary cause of crop failures in India. It has been estimated that about 8.6 million ha of land is affected by salinity in India (Pathak, 2000).These stresses are increasing drastically these days because of pollution, declining availability of good quality water and land degradation.

Cereals constitute a major source of food for the human population of the world. Important cereals in day to day use are rice, wheat and millets. Of the all the millets *Eleusine* contains more percentage of different nutrients as compared to rice and wheat. It is a rich source of calcium, magnesium and potassium. *Eleusine* is nutritionally very rich cereal and is a staple food for poor and invalids (Panda,

1999). Due its nutritional superiority and requirement by poor people production needs to be improved. Previous work has proved *Eleusine* to be a favorable material for tissue culture studies. *Eleusine coracana* belonging to family Poaceae will be used as a model plant in the present study.

Eleusine coracana mainly growing in dry condition faces heat, salinity and heavy metal stress which may cause negative effect on its yield and adequate work has not been done to evaluate the effects of stresses and requires immediate attention by the researchers. The present study will be therefore undertaken to study stress tolerance in *Eleusine* by variations in the tissue culture media and evaluating performance of field grown plants under similar conditions and thus proposing solutions to the farmers. Stress tolerant plants can also be developed by breeding and by transgenesis which are complex processes. (Babu *et al*, 2007) Tissue culture techniques offer an easy and important tool in developing stress tolerant variants (Nabors and Dykes, 1985.).

E. *coracana* is considered to be of Indian or African origin. It is widely cultivated in tropical Asia, and East Africa cultivated on rainy slopes and upland areas of Himalayas up to 2,300 m elevation. It is one of the best suited crops for dry farming, generally grown rain fed. Ragi is very adaptable and thrives at higher elevations than most other tropical cereals. It is cultivated on soils ranging from rich loams to poor shallow upland soils. In India it is grown on black cotton soils, but thrives on red lateritic loams. Ragi stands salinity better than most cereals

Eleusine is a tufted annual grass that grows to a height of 210-620 mm tall. The leaf blades are shiny, strongly keeled, and difficult to break and are 220-500 mm long and 6-10 mm wide. The leaves and culms are typically green in color. It has an exceptionally strong root system. The culms and the leaf sheaths are prominently flattened. The spikelets are 5-8 mm long and 3-4 mm wide. The grains are globose.

Objectives

Food productivity is decreasing drastically mainly because of abiotic stress factors like salinity, drought, heavy metals, pesticides, etc. Growing condition can be redesigned to better scope with detrimental effects of stresses. Agriculture produce can be increased to a great level .The traditional breeding approaches employed to develop crop varieties best suited to environmental conditions have the limitation of space, labor and time which can be overcome by including in vitro studies taking the same objectives. *In vitro* studies require comparatively less time and space and will help us developing stress tolerant plants.

Eleusine mainly grows in dry condition and faces salinity and heavy metals stress, limiting its productivity. To increase the production, ways and means have to be developed to overcome stresses. This field of research is still unexplored and needs prior attention. The work in the field of stress management has been done mainly on wheat using either traditional breeding methods or transgenesis but these are cumbersome and time consuming, Tissue culture techniques provide an important tool in developing stress tolerance somaclonal variants, The present study would therefore be undertaken to study the in vitro behavior of Eleusine on artificially created stress environment. This would help in finding an easy and approachable solution for the problem. The study would therefore be planned with the following objectives:

- To develop an efficient protocol for plant regeneration system *(in vitro)* using callus derived from different explants like seeds, mature and immature embryos, young leaves, shoot apex.
- To study the influence of various stress inducers on *Eleusine coracana* tissue culture and establishment of the detrimental level of each.
- To optimize the culture condition by adding nutrients with an objective for minimizing stress injuries and maximizing the yield.
- To study the field performance of *Eleusine* plant with the same conditions as provided under *in vitro* condition.
- Biochemical estimation of protein from plants under stress and plant free from stress

Review of Literature

Plant tissue culture is in great use to fulfill the objective of improving the yield, which is not possible through the conventional methods of breeding. Plant biotechnology consists of a variety of techniques, designed to genetically improve and /or exploit living systems or their components for the benefit of man. The present review of literature will focus on tissue culture work done in the family Poaceae and study of effect of abiotic stress on regeneration. Improvement of plants through tissue culture techniques is desired because classical breeding is generally slow, laborious and time consuming and is constrained by the limited genetic pool.

Murashige and Skoog formulated the MS media for tobacco taking into consideration the nutritional composition of tobacco plants .This gives an idea that nutritional requirement for different plant is different and would have an effect on the growth. Changing the nutrient composition of the media can alter the requirements and percentage of the explants responding by altering enzyme activity (Murashige and Skoog, 1962; He *et al*., 1989, 1991; Preece *et al*., 1995; Poddar *et al*., 1997; Samoylov *et al*., 1998) Thus variation in the tissue culture media is expected to vary the response of plants to stress conditions also. The same principle may help us in achieving resistance against stress under *in vivo* conditions.

Nutrients have also been known to replace the PGR in tissue culture media (Preece *et al*., 1995) Poddar *et al*, (1997) studied the effect of different ammonium nitrate, used as macronutrient in the MS medium, for plant regeneration from embryogenic callus of *Eleusine coracana* in the presence and absence of NAA the regeneration hormone. Results showed that addition of higher concentrations of ammonium nitrate can substitute for the growth regulator requirement in the medium for plant regeneration

Kothari *et al*, (2004) also studied that growth and morphogenesis of plant tissues under *in vitro* conditions are largely influenced by the composition of the culture media. Effects of different inorganic nutrients like $ZnSO_4$ and $CuSO_4$, on callus induction and plant regeneration, of *Eleusine coracana in vitro* were also examined. Primary callus induction without $ZnSO_4$ resulted in improved shoot formation upon transfer of calluses to normal regeneration medium.

Abiotic stresses, specially salinity, heavy metals and pesticides are the primary causes of crop loss. These stresses are increasing because of declining availability of good quality water and land degradation. *Eleusine* is grown in hostile environmental conditions facing stresses. Physiological and biochemical mechanisms of plant abiotic stress resistance are discussed by (Bohnert *et al*., 1995; Ngyugen *et al*., 1997; Zhang *et al*., 2000). Farrukh (2004a, b) also studied the organic solute accumulation of salt tolerance on wheat cultivars

Addition of ABA to the induction medium containing 200mM NaCl is reported to enhance the acquired tolerance of finger millet seedling. Uma *et al*, (2009) this study also indicated that the synthesis of stress specific proteins is also co related with the observed acquired tolerance. These results are in accordance with the previous results of Aarti *et al* (2002) and Lalit (2002). Even Jayaprakash *et al*. had the same observation in (1998). They found that in *Eleusine*, salt tolerant species have higher LEA 2 and LEA3 proteins as compared to normal plants. Divakaran Sastry and Gupta, (2009) studied stress conditions in petridishes. It was found that salt and high temperature stress reduced the growth this might be due to disruption of ionic equilibrium of the cell. Stress-induced changes in the cytosolic concentration of Ca was reported by Knight,(2008) Effect of NaCl at 2%, 4 %, 6% and 8% were tested by Babu *et al,*(2007)under *in vivo* and *in vitro* conditions which gave specicific results. Inspite of these searches a need is felt to overcome the problem of stress. At higher concentration, calli lost water content and represented degenerated tissue and even died .Similar callogenesis response under NaCl stress in wheat cultivar was observed by Mahmood and Quaraishi (1985) and in rice by Abbas *et al*, (1983).

Ghannadha *et al,* (2005) studied the relationship between different traits and salt tolerance in wheat. Different concentration of NaCl have no effects on either callus fresh weight or callus number but increased salt stress decreased the callus size. Variation of K was also related to tolerance or sensitivity of cultivar. Tolerant cultivars also showed higher germination percentage, coleoptiles length and root length.

Sanghamitra *et al*,(2001) studied chromium and nickel tolerance in *Echinochloa colona* L. through callus culture derived from leaf base, leaf tip and mesocotyl explants on Murashige and Skoog (1962) medium supplemented with 0.5 mg /l, BA 3.0 mg /l , 2,4-D and 1.5 mg /l chromium plus 2.0 mg $\cdot L^{-1}$ nickel. This study helped in the selection of metal tolerant plants which might be used for phytoremediation programmes. Verma and Dubey (2001) also studied the effects of cadmium on the content of starch and sugars, and changes in the activities of the enzymes of sugar metabolism in growing seedlings of rice (Oryza sativa L.) cultivars Ratna and Jaya. Cd induced increase in the sugar content was greater in shoots than in roots.

Agrawal and Sharma (2005) cultured *Holarrhena antidysenterica* L .nodal explants on Murashige and Skoog's (MS) medium augmented with N^6-benzyladenine (BA) alone (control) or supplemented with different concentrations of $CdCl_2$, $CuSO_4$, $Pb(NO_3)_2$ and $ZnSO_4$. The maximum morphogenic response in terms of average shoot number was seen in control. $ZnSO_4$ proved to be less inhibitory in comparison to $CuSO_4$,$Pb(NO_3)_2$ and $CdCl_2$,Daud et.al., (2008) also studied the toxic effect of cadmium (Cd) at various physiological and morphological parameters of the root and whole plant in both transgenic cotton cultivars and their relative wild cotton genotype respond differently towards the Cd toxicity.

Weiqiang *et.al*., (2005) analyzed toxicity of essential (Cu^{2+} and Zn^{2+}) and non-essential heavy metals (Hg^{2+}, Pb^{2+} and Cd^{2+}) on seed germination and seedling growth in the model species, *Arabidopsis*. Results showed that seedling growth was more sensitive to heavy metals (Hg^{2+}, Pb^{2+}, Cu^{2-} and Zn^{2+}) in comparison to seed germination, while Cd^{2+} was the exception that inhibited both of these processes at similar concentrations

Traditional and modern breeding approaches were always common methods of overcoming stress. However genetic transformation for protecting crop plants from major stresses is also gaining impetus (Langridge *et al.,* 2006; Thompson *et al*., 2007; Sreenivasulu *et al*., 2007; Witcombe *et al*., 2010). Looking to the economics of *Eleusine,* it is now time to shift to some easy approach and tissue culture may be a promising option.

Methodology

An important plant: *Eleusine coracana* (L.) Gaertn of family Poaceae was taken as the model plant for the study. Seeds are argonomically superior and released variety (PR 202) of Ragi would be procured from **Agricultural University, Banglore.**

***Eleusine coracana* (L.) Gaertn**
Common Name : Finger millet
Vernacular Name: Ragi

The medium used in the present study would be MS (Murashige and Skoog, 1962) medium supplemented with various hormones. The amount of sucrose would be 3%, agar 0.8% and the pH would be adjusted to 5.7-5.8 using 1N HCL or NaOH. Medium would be sterilized in autoclave at 1.06 kg/cm^{2}for 15 minutes.

Primary Callus Induction and Plant Regeneration

For primary callus induction different explants such as seeds, mature and immature embryos, young leaves ,shoot apex were inoculated to MS medium with different hormonal combinations for callus induction.(1 mg 2,4-D; 2 mg 2,4-D, 4 mg 2,4-D, 0.5 mg Kn + different concentration of 2,4-D). Explants would be taken from *in vitro* grown seedling of ***Eleusine coracana*** (var 202). Aseptic manipulation would be done under laminar air flow cabinet, previously irradiated with U.V rays for 50-60 minutes. After inoculation the cultures would be placed in culture chamber with a temperature and humidity controller and a photo timer. Callus induced in each case would be observed. Fresh weight and dry fresh weight of each of the callus would be taken in each case. The callus would then be transferred on proliferation medium which generally contains hormones used for callus induction but at much lower level. The embryogenic callus so developed would be transferred to MS medium supplemented with regeneration hormones (different concentration of GA_3 and NAA) Same amount of embryogenic callus would be transferred to each flask and number of shoots would be counted in each case and the combination which would give maximum number of shoots would be taken as model protocol in the future study.

Effect of various stress inducers on growth

Study of the *in vitro* behavior of *Eleusine* on artificially created stress environment by addition of stress causing agents such as NaCl, heavy metals like Ni, Al and Pesticides (0%, 1%, 2%, 3%, 4% and 5%) to the callus induction medium of *Eleusine* would be done and observe the number of shoots regenerated from this callus (developed under stress condition) on normal regeneration medium (control). From these experiments detrimental level of each stress inducer will be established (M1 medium).

Effect of various nutrients on callus induction

In the second set of experiment we would add various nutrients expected to overcome stress at different concentration, to the induction medium. i.e. callus induction medium with detrimental level of the stress causing agent (M2 media) and observe the number of shoots regenerated on normal regenerated medium from these calli.(developed under supposedly stress relieved condition). Final observation (no. of Shoots) would be taken after 6 weeks and result would be interpreted statistically. The variation in the number of shoots in both experiments would enable us to evaluate the stress relieving potential of different nutrients and their optimal level required to perform the task. Same amount of embryogenic callus would be transferred to each flask with five replica of each type. Observation would be taken regularly for the growth of the callus to study the effect of stress on callus induction. After 4 weeks of callus induction fresh weight of each of the callus would be taken.

Study of field performance -Detrimental concentration of each stress causing agent would be added to the soil .The optimal level of stress relieving agent determined, would also be added and the performance of these plants would be studied.

Biochemical estimation -Protein would be isolated from plants under stress and with stress relieved condition and estimated biochemically using spectrophotometer and identified using SDS-PAGE gel electrophoresis.

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